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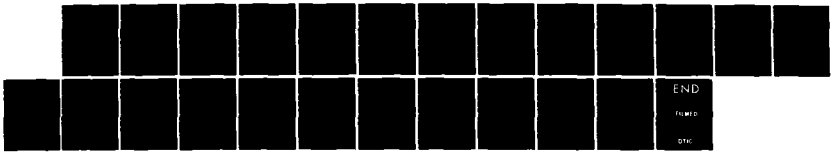

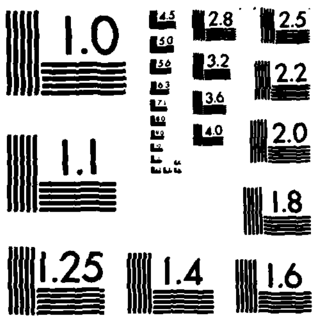

MICROCOPY RESOLUTION TEST CHART
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Tuskegee Institute

TUSKEGEE INSTITUTE
ALABAMA 36088

SCHOOL OF VETERINARY MEDICINE
DEPARTMENT OF MICROBIOLOGY

January 30, 1985

Dr. Jeannine A. Majde
Program Manager
Cellular Biosystems Program
Office of Naval Research
Arlington, VA 22217

Dear Dr. Majde:

Please find enclosed copies of the Final Report for ONR Contract #N00014-83-K-0566, NR612-024. I regret the delay in filing which was due to problems in interpretating DOD Form #1473. I hope this meets with your approval.

Copies of the report have been sent to the proper agencies as you requested.

Thanks for your help and cooperation.

Very truly yours,

E. M. Jenkins
Principal Investigator

EMJ/p

cc: Mr. Michael Karp
Defense Technical Information Center

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Since this was primarily a research training grant, the main emphasis during the initial stages of the program was concerned with the orientation of graduate students to a wide range of techniques used in basic and applied immunological research. The research grant provided support for graduate students on a Masters' Degree Program in Microbiology, all of whom are in good academic standing. Two of these students will complete their work this spring '85 and the other four will finish their work during the fall semester of this same year. It is unfortunate that these students will no longer be supported by this grant in completing their work. However, they have expressed their gratitude with the realization that without this support they would not have been able to acquire this training.

In addition to the regular graduate training program, these students were able to acquire a variety of conventional and modern skills provided by outside lecturers, workshops, symposiums, and scientific meetings. Some of these students have already presented papers at scientific meetings and all will do so at a major convention before the

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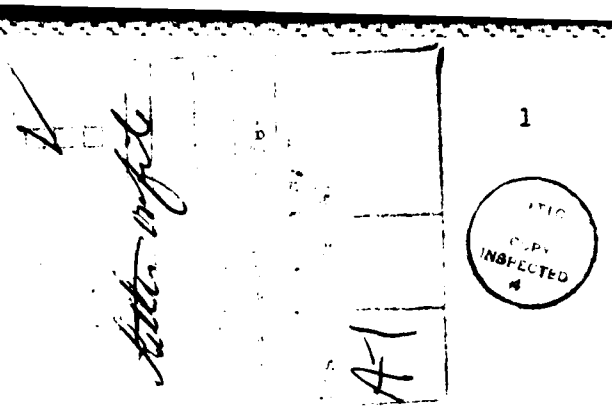

A. Purpose of the Research

The purpose of the grant is to provide the curriculum and research for graduate students to acquire knowledge and technical competency in an understanding of basic and applied principals in immunology. Therefore, the support requested in this grant is primarily intended to: (1) Develop our research capabilities through training of graduate students to become competent researchers as well as expand current research laboratory space; (2) Expand the current research projects of several faculty members; and (3) Establish a base for a centralized electron microscopy laboratory. This grant will not only increase our effectiveness in the graduate research participation program but will also help the faculty develop their professional expertise through research. In essence, the grant will greatly strengthen our graduate research program, which suffers severely due to the limited funds currently available for graduate study and will also provide a strong base for manpower training as well as a potential source for Naval personnel. We will continue work on the development of improved methods for identification and characterization of allergens involved in hypersensitivity reactions. We believe that research in these areas will complement the Naval research program in microbiology and will not duplicate research being supported by other government agencies.

Key Words

Graduate students, immunology, electron microscopy, hypersensitivity reactions, aging, Naval research program, allergens.

B. Abbreviated Objective and Relevance to Navy Needs

In the development of improved methods for identification and characterization of allergens involved in hypersensitivity reactions, the research will address itself to the refinement of currently used immunologic procedures and develop new ones for rapid identification of allergens involved in hypersensitivity reactions. The allergens will be selected from a broad spectrum of the environment where Naval personnel are likely to be exposed. Tests to demonstrate cell-mediated immunity (CMI) will be refined for rapid identification of immediate and delayed-type hypersensitivity (DTH) allergens in the environment as well as of food and clothing. An attempt will be made to improve currently used methods for the detection of specific antibody (IgE, IgG) involved in allergic reactions including a battery of tests such as the radioallergosorbent test (RAST), radial immunodiffusion assay, enzyme-linked immunosorbent assay (ELISA) and immunofluorescence. Tests to demonstrate CMI will all be developed and their usefulness as diagnostic tools determined. Small laboratory animals (guinea pigs, mice) as well as large domestic animals (dogs, swine, chickens and goats) will be used as experimental models. The clinical signs, symptoms and level of constituent involved in allergic reactions will be correlated with those seen in humans through the cooperation of physicians at the community hospital located on campus; however, human subjects will not be used in the initial study. The research is expected to provide information that may be useful in screening as well as assist in the therapeutic treatments of Naval personnel subjected to allergenic hazards of the environment.

C. Progress of Students

Much of the first year of the research training grant was devoted to the development of an acceptable graduate program and getting new students oriented to techniques generally used in the laboratory. Four new students were assigned to the training program in addition to two others who were already aboard.

The study-work involved courses aimed at providing a basic background in biochemistry, immunology, physiology, as well as radiation biology that will enable the students to carry out the research as planned. The new students have done well with their academic requirements and are at about the midway point in completing them.

Various techniques have been implemented by older graduate students in an effort to enhance the technical skills of the new graduate students. These newer students have actually acquired laboratory skills by actively participating in the work of other students as well as their own.

The graduate students had the opportunity to attend two scientific meetings last year--the Southeastern Immunology Conference in Atlanta, Georgia and the Southeastern Branch of the American Society for Microbiology, held in Tuscaloosa, Alabama. This fall, two students (Sharlene Holmes and Ian Blackwell) will present data related to their research findings at the Southeastern Immunology Conference, Atlanta, Georgia. Jerry Dillon will present a paper at the Fifth Biennial Symposium, 1890 Land Grant Colleges, Dallas, Texas. Other students will present papers at the Southeastern Biology Conference in Columbia, South Carolina in the Spring, and at the ASM National meeting in Las Vegas, Nevada.

D. Research Progress

1. Development and refinement of diagnostic tests to demonstrate humoral antibody response hypersensitivity reactions. During the summer and early fall more than 100 serum samples were collected from dogs admitted to our Veterinary Clinic and/or the local county dog pound. Most of these dogs showed clinical signs of atopic dermatitis that were clinically classified as follows: (1) Severe (4+) excoriations requiring administration of prednisolone for control of the intense pruritus associated with skin lesions; (2) Moderate (3+) excoriations with alopecia and secondary lesions but not requiring prednisolone to relieve associate pruritus; (3) Slight (2+) evidence of pruritus resulting in mild secondary excoriations identifiable only by salivary staining of the pelage or minimal alopecia in areas which had been scratched, rubbed or licked and/or a vesiculopustular eruption in areas commonly associated with atopic dermatitis (i.e., face, eye, nostrils, and ears). Sera were likewise collected from dogs that showed no clinical signs of the disease and was used as control serum.

a. Methods of Assay

(1) RAST

A modification of Halliwell and Kunkle's radioallergosorbent test (RAST) employing common ragweed (pharmacia) allergen disks with fractionated anti-IgE was used.[1] The results were recorded as average counts per minute

on duplicate samples to those for the IgE-negative control; values < 2.0 were considered negative.

(2) ELISA

An enzyme-linked immunosorbent test (ELISA) was developed employing ragweed extract (pharmacia) as antigen and a conjugate prepared with dog partially purified IgE conjugated to horseradish peroxidase enzyme. A serum showing an optical density reading (ODR) of $\geq$ .1 above the background reading was considered positive.

2. Tests to demonstrate cell-mediated immunity in dogs affected with atopic dermatitis.

a. Method of assay

Dogs were selected at random from those showing clinical signs of atopic dermatitis as indicated above.

(1) Numerical distribution of T-total, T-active and B Lymphoid population.

(2) Chemotaxis of blood neutrophiles.

(3) Skin tests or passive cutaneous anaphylaxis.

A depressed level of T cells with receptors of low avidity for sheep red blood cells, T-total, has been reported in human cases of rhinitis.[2] Depression of the lymphocyte count with receptors for the FC fragment of IgG (T_G suppresssor of B cellular differentiation induced by pokeweed mitogen) in subjects with seasonal pollenasis was also reported. Based on this information we performed studies to determine the level of T and B cell populations in dogs affected

with atopic dermatitis hoping that it may offer some aid as a diagnostic tool for this condition, both in dogs and humans, according to methods previously described.[2]

(1) To count the number of T-total lymphocytes, aliquots of a suspension of lymphocytes (4×10^6 cells/ml) were mixed and incubated for 5 minutes at 37°C. After centrifugation for 5 minutes at 200g they were left overnight at 4°C. A total of 200 cells were counted and lymphocytes with three or more attached red blood cells were considered to be positive.

To count the T-active population, 0.1 ml of lymphocyte suspension (4×10^6 cells/ml) and 0.1 ml fetal calf serum (inactivated), which had been absorbed with SRBC, were mixed and incubated for 60 minutes at 37°C. After adding 0.1 ml SRBC, the suspension was centrifuged at 200 g for five minutes before very gently resuspended. The readings were carried out as in the case of the T-total lymphocytes.

To enhance the resulting assay of both T-active and T-total lymphocytes, SRBC were treated with a 2-aminoethylisothiouronium bromide (AET), neuramenidase or ficoll 400 prior to adding to the test system.

For the study of the lymphoid population, 0.1 ml of lymphocyte suspension (4×10^6 cells/ml) and 0.1 ml antiserum dog anti-Ig fragment F $(Ab)_2$ labelled with fluorescein were mixed, incubated for 45 minutes at 4° C and then washed three times with PBS. The cellular sediments were then placed on a microscopic slide on which

a reading of the cells was taken with a fluorescent aus Jena microscope (200 fluorescent and non-fluorescent cells were counted).

Stimulation with PHA and PWM. An (0.1 ml) aliquot of lymphocyte suspension was adjusted to 1 x 10^6 cells/ml and distributed in different wells of a flat-bottom microtiter plate. We then added 0.1 ml of a solution of PHA-M (Gibco) in culture medium RPMI 1640 at a concentration of 10 mg/ml or PWN (Gibco) at a concentration of 10 mg/ml to some of the wells.

All wells contained 0.1 ml of RPMI 1640 with 20% fetal calf serum. The plates were then incubated at 37°C in a humid atmosphere at 5% CO_2 for 92 hours. Eighteen hours before completion of the culture, 0.2 uCi of thymidine ^3H was added to each well. The cells were collected on a fiberglass filter and then transferred to a 5 ml vial of scintillation liquid and read on a Beckman Counter. The results were recorded as counts per minute (CPM) and the response index (RI) was expressed by dividing the means obtained in the presence of PHA or PWM and the control well.

(2) Chemotaxis. Chemotaxis was carried out by the Boyden Chamber procedure using a 3 μm pore size 13 mm diameter polycarbamate filter (Unipore, Bio-Rad). A mixture of short and giant ragweed extract (pharmacia) was used as the chemoattractant. Polymorphs (PMN) were shocked out of peripheral blood with 0.87 NH_4CL after which Hank's balanced salt solution (HBSS) with Ca^{++} and Mg^{++} (2X) was added to stop the reaction. After several washings in HBSS the chemoattractant (10, 100, or 1000 units) was placed in the lower compartment of the chamber at the level of the lips. The membrane was then placed in

position with glistening side up. The white top of the chamber was then placed in the top of the chemotactant solution and filters using caution not to tear the membrane. The PMNs (a 0.2 ml suspension containing 1 x 10^7 cells) was placed in the top compartment of the chamber and the chamber was incubated at 39°C for 90 minutes under 5% CO_2 tension. The membranes were then fixed to a microscopic slide by heat, stained and counted under 1000 x oil immersion magnificate. An ocular micrometer was used for counting and 20 microscopic fields were counted.

(3) Skin tests. The dogs, unsedated, were skin tested with 0.1 ml of serial dilution (100 µg, 10 µg, 1 µg, 100 ng, 1 mg) of ragweed extract injected intradermally into the shoulder flank immediately followed by 0.5% Evan's blue dye injected intravenously. The skin reactions were recorded over 20 minutes. Only skin reactions showing a bluing and a diameter of greater than 5 mm were considered positive.

E. Progress and Principal Accomplishments

Serologic examination of the sera of dogs with naturally occurring dermatitis by the ELISA detected 10 (83%) of 12 dogs affected with severe atopic dermatitis, 9 (64%) of 14 dogs affected with moderate atopic dermatitis, and 4 (33%) of 12 dogs affected mildly with the condition, Table 1. Two dogs of 12 thought to be normal gave a positive reaction. With respect to the RAST, only 3 (25%) of the twelve dogs tested with severe atopic dermatitis were positive, while 2 moderately affected were also positive. There were no positive reactors in the mildly affected or normal dog group.[5]

There was a significant difference ($P < 0.05$) in the T-total rosettes of SRBC treated with AET and those not treated of lymphocytes from dogs affected with atopic dermatitis, Table 2. However, there was no significant difference ($P > 0.05$) in T-total rosettes of SRBC treated with AET and NMD. AET was also superior ($P < 0.05$ to both NMD treated SRBC and those not treated of the T-active lymphocytes.

The chemotaxis of neutrophiles of dogs affected with atopic dermatitis was significantly greater ($P < 0.05$) in dogs tested with 100 and 1000 units of the chemotactant than that of the medium control. However, there was no significant difference ($P > 0.05$) in those atopic dermatitis dogs treated with 10 units of ragweed and the media control, Table 3.

When the chemotactic responses of two strains of mice immunized with ragweed allergen were tested against 100 and 1000 units of the homologous allergen the chemotaxis was significantly suppressed as compared to the control mice (unimmunized), Table 4. The chemotaxis of N:GP(S) mice immunized with ragweed allergen in combination with cyclophosphamide

was significantly more suppressed, overally, than those of the strain SJ8/N-bg.

The B lymphocyte population of mice was reduced about 1/3 in both N:GP(S) and SJ8/N-bg strains of mice immunized with ragweed allergen and less than ½ in the mice given ragweed allergen in combination with cyclophosphamide, Table 5. Likewise, the humoral antibody response was suppressed in both cases where cyclophosphamide was given.

There was a significant difference ($P < 0.05$) in the T-total lymphocytes of mice immunized with ragweed antigen and those of the controls (unimmunized mice) for both the N:GP(S) and SJ8/N-bg strains of inbred mice, Table 6. However, there was no significant difference in th response of T-active lymphocytes. With respect to lymphocyte stimulation there was a significant increase ($P < 0.05$) in PHA stimulation of lymphocytes in both strains of mice. However, there was no difference in the lymphocyte stimulation index of the ragweed immunized mice of both strains and the unimmunized controls. Likewise, there was no difference in the humoral antibody titer of the two strains of mice.

The mitogenic stimulation of lymphocytes of dogs moderately affected with atopic dermatitis was significantly increased over the media control for both PHA and PWM, Table 7. By the same token, there was a significant decrease ($P < 0.05$), overally, when these results were compared with two normal dogs. A good correlation existed between the direct skin test and the lymphocyte stimulation index since 4 of the 5 dogs reacted, ranging from 1^+ - 3^+ in the 100 and 1.0 µg conc. dose.

The chemotactic and rosetting properties of neutrophiles and lymphocytes, respectively, of dogs as shown in Table 8, indicates a significant

difference ($P < 0.01$) in the response of dogs treated with 1000 units of allergen compared to normal dogs. However, there was no significant difference ($P > 0.05$) in the response of atopic dogs treated with the 10 or 100 conc. of the allergen. Likewise, there was no significant difference ($P > 0.05$) in the T lymphocytes of atopic or normal dogs as determined by rosetting with AET SRBCs.

F. Usefulness of Findings

Based on a variety of immunologic tests to detect humoral antibody (IgG and IgE) and CMI responses in dogs with natural occurring allergic reaction or mice immunized with ragweed antigen the following assumptions can be made:

1. The ELISA appears to be potentially useful as a serologic test for the detection of atopic dermatitis.
2. At the present stage of development the quantitation of T-lymphocyte population as determined by the rosetting technique of SRBCs in the mouse and dog models does not appear to be useful as a screening test for atopic dermatitis since it doesn't differentiate between the so called normal and atopic animal.
3. Chemotaxis appears to be useful as a diagnostic tool in the mouse model for atopic dermatitis since CMI appears to be depressed in two strains of immunized mice when compared with controls.
4. In the dog model chemotaxis is generally increased over normal dogs and can be considered to be potentially useful as a diagnostic tool.
5. Lymphocyte stimulation in atopic dogs appears to be significantly depressed when compared to normal dogs and may have some potential as a diagnostic tool. The direct skin tests correlate well with the lymphocyte stimulation test. However, these reactions were only weakly positive. More tests will have to be carried out before conclusions can be made.

G. Publications:

Dillon*, Jerry, Sharlene Holmes*, Jonathan Moseley* and E. M. Jenkins. Comparison of Mecadox with Bacitracin in the Prevention of Clinical Signs of Swine Dysentery. Fifth Biennial Research Symposium, Research Director, 1890 Land Grant Colleges and Tuskegee Institute, Dallas, Texas, Oct. 22, 1982.

Moseley, Jonathan and E. M. Jenkins. Comparison of Mecadox with Bacitracin for Controlling Swine Dysentery. Minority Biomedical Research Symposium, Washington, D. C., April, 1984.

Hawkins*, Diane, and E. M. Jenkins. Effect of Levamisole in the Development of Cellular Immunity to T. hyodysenteriae. Minority Biomedical Research Symposium, Washington, D. C., April, 1984.

Blackwell*, Ian. Allergic Dermatitis in Dogs. Veterinary Symposium, Tuskegee Institute, 1983.

Froe, D. L. and E. M. Jenkins. Mecadox-Modulation of the Immune Response to T. hyodysenteriae. 8th International Veterinary Symposium, Ghent, Belgium, August 21, 1984.

Nash, Stephen. Secretory Immunity in Swine Dysentery. Master's Thesis. Tuskegee Institute, August, 1984.

Jenkins, E. M., P. H. Klesius, John Alak* and Sharlene Holmes*. Application of Chemiluminescence to the Diagnosis of Swine Dysentery. Fifth Biennial Research Symposium, Research Director, 1890 Land Grant Colleges and Tuskegee Institute, Dallas, Texas, Oct. 22, 1984.

William, Erwine B. Membrane Filter: Fluorescent-Antibody Technique for Rapid Identification of Salmonella in Drinking Water. Master's Thesis, Tuskegee Institute, 1983.

Blackwell*, Ian, Sharlene Holmes*, Jerry Dillon* and E. M. Jenkins. The Nature of Chemiluminescence Response in Swine-Dysentery Affected Animals. Southeastern Immunology Conference, Atlanta, GA, November 1-4, 1984.

Holmes*, Sharlene, Ian Blackwell*, Jerry Dillon*, Pamela Guy* and E. M. Jenkins. Chemotaxis of Mice Peritoneal Cells to Soluble Antigens of Treponema hyodysenteriae. Southeastern Immunology Conference, Atlanta, GA, Nov. 1, 2, 1984.

*Graduate students participating in many research projects.

TABLE 1. COMPARISON OF SEROLOGIC RESULTS OF DOGS DURING DIFFERENT STAGES OF NATURALLY OCCURRING ATOPIC DERMATITIS

STAGE OF CONDITION	NO. ANIMALS TESTED	NO. AND % ANIMALS POSITIVE	
		ELISA	RAST
SEVERE	12	10 (83%)	3 (25%)
MODERATE	14	9 (64%)	2 (14%)
MILD	12	4 (33%)	0
NORMAL	12	2 (16%)	0

TABLE 2. COMPARISON OF ROSETTING OF LYMPHOCYTES OF DOGS AFFECTED WITH ATOPIC DERMATITIS USING DIFFERENT TREATMENTS OF SRBC

	AET+ ROSETTE %		NMD++ ROSETTE %		NO TREATMENT %	
ANIMAL #	T-ACTIVE	T-TOTAL	T-ACTIVE	T-TOTAL	T-ACTIVE	T-TOTAL
10C[A]	6	12	2	2	2	4
12P[B]	8	8	4	4	3	2
14C	5	16	4	4	2	2
16P	9	17	4	5	3	2
20C	5	18	5	7	4	3
24P	6	16	3	8	5	4
30C	8	19	4	7	3	4
36P	2	15	3	9	2	3
	6.13±2.23*	15.13±3.56	3.63±0.92	5.75±2.37	3.0±1.07	3.0±0.93

*MEAN COUNT OF 200 LYMPHOCYTES

+AMINOETHYLISOTHIOURONIUM BROMIDE 4 ML OF .125M/1 ML PACKED SRBC

++NEURAMINIDASE, 10UT/0.5 ML PACKED SRBC

[A]SAMPLE OBTAINED FROM DOGS IN VETERINARY CLINIC, TUSKEGEE INSTITUTE

[B]SAMPLE OBTAINED FROM DOGS OF MACON COUNTY DOG POUND

TABLE 3. CHEMOTACTIC RESPONSE OF NEUTROPHILES OF DOGS AFFECTED WITH ATOPIC DERMATITIS TO DIFFERENT CHEMOTACTANTS

DOG NUMBER	SEX	NEUTROPHILES/20 RANDOM FIELD				
		RPMI MEDIA*	POSITIVE SERUM CONTROL**	RAGWEED CONC. (UNITS) 10	100	1000
4C	F	170	340	170	400	425
7D	M	160	480	140	413	396
8C	M	111	380	132	350	526
18C	M	162	248	141	436	379
20D	F	136	426	113	356	506
$\bar{X} \pm SD$		147.8±24.17	374.8±87.75	139.2±20.56	391±37.07	446.4±65.15

* RPMI - 1640

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TABLE 4. CHEMOTACTIC RESPONSE OF NEUTROPHILES OF TWO STRAINS OF MICE SENSITIVE TO RAGWEED ALLERGENS

TREATMENT	STRAIN OF MICE					
	N:GP(S)*			SJ8/N-BG**		
	CHEMOTACTANT					
	RAGWEED ALLERGEN (UNIT)		MEDIUM CONTROL	RAGWEED ALLERGEN (UNIT)		MEDIUM CONTROL
	100	1000		100	1000	
RAGWEED ALLERGEN	326+	253	516	405	404	296
RAGWEED ALLERGEN + CYCLOPHOSPHAMIDE	369	295	420	159	230	239
CONTROL	253	687	269	510	945	320

* NIH GENERAL PURPOSE

**SELECTIVE FOR ALLERGY RESEARCH

+ AVERAGE TOTAL COUNT

TABLE 5. MEAN B LYMPHOCYTE POPULATION HUMORAL ANTIBODY RESPONSE OF MICE SENSITIZED WITH RAGWEED ALLERGEN AND NORMAL MICE

MICE (STRAIN) AND TREATMENT	% B LYMPHOCYTES	ELISA+
N:GP(S)*		
RAGWEED ++	8	64++
RAGWEED + CYCLOPHOSPHAMIDE	4.5	8
CONTROL	12	4
SJ8/N-BG**		
RAGWEED	6	128
RAGWEED + CYCLOPHOSPHAMIDE	3.5	16
CONTROL	9	4

* NIH GENERAL PURPOSE

**SELECTIVE FOR ALLERGY RESEARCH

+ MOUSE IgG CONJUGATE

++1000 UNIT RAGWEED ANTIGEN

TABLE 6. COMPARISON OF ROSETTING OF LYMPHOCYTES, LYMPHOCYTE STIMULATION, AND HUMORAL ANTIBODY OF MICE IMMUNIZED WITH RAGWEED ANTIGEN*

MICE (STRAIN AND TREATMENT)	LYMPHOCYTE ROSETTING % T-ACTIVE	T-TOTAL	STIMULATION INDEX PHA	PWM	MEDIA	ELISA TITER
N:GP(S)						
RAGWEED	16	26	32.4 ± 42	16 ± 3.4	9.4±2.3	16
CONTROL	14	22	16.2 ± 6	18 ± 4.6	6±4.5	4
SJ8/N-BG						
RAGWEED	12	18	37 ± 7.6	19 ± 3.4	8±4.5	32
CONTROL	14	25	17 ± 4.8	16 ± 4.7	6±4.2	4

*1000 UNITS

TABLE 7. CELLULAR IMMUNE RESPONSE IN DOGS SHOWING CLINICAL SIGNS OF ATOPIC DERMATITIS AND NORMAL DOGS

DOG	STIMULATION INDEX			DIRECT SKIN TEST ANTIGEN CONC. (µG)				
	MEDIA	PHA	PWM	100	1.0	1	.01	.001
14C*	16 ± 4.6	43 ± 1.4	25 ± 3.2	2^+	0	0	0	0
20P**	15 ± 3.7	42 ± 1.9	27 ± 9.2	-	0	0	0	0
30C	11 ± 6.3	36 ±18.2	22 ± 7.8	3^+	2^+	0	0	0
36P	12 ± 3.4	28 ±16.6	27 ± 9.5	0	1^+	0	0	0
40+	10 ± 2.6	42 ±12.4	32 ± 4.8	0	0	0	0	0
45+	13 ± 1.2	46 ±25.6	23 ± 3.7	0	0	0	0	0

* CLINIC DOG

**COUNTY POUND DOG

+ NORMAL DOG

TABLE 8. A COMPOSITE COMPARISON OF CHEMOTAXIS AND ROSETTING PROPERTIES OF DOGS AFFECTED WITH ATOPIC DERMATITIS AND NORMAL DOGS

DOGS	% LYMPHOCYTE ROSETTING**		CHEMOTAXIS (TOTAL CELL COUNT) CONC. OF RAGWEED			
	T-ACTIVE	T-TOTAL	10	100	1000	CONTROL MEDIA
ATOPIC DERMATITIS*	9.4 ± 4.2	24.2 ± 3.6	1380	2125	463	2877
NORMAL*	8.4 ± 4.3	7.6 ± 2.4	192	404	487	525

*(N = 8) DOGS

**CARRIED OUT WITH AET SRBC

END

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DTIC